Effect of pH on continuous biohydrogen production from a mixtureof End-of-Life Dairy Products (EoL-DPs)

K.P. Stavropoulos[1] and M. Kornaros[1,*]

[1]*Laboratory of Biochemical Engineering & Environmental Technology (LBEET), Department of Chemical Engineering, University of Patras, 1 Karatheodori str., 26504 Patras, GREECE*

*Presenting author e-mail: kornaros@chemeng.upatras.gr

Tel: +30 2610 997418, Fax: +30 2610 993070.

Abstract

The aim of the present study was to determine the optimal pH value for treating a mixture of End-of-Life Dairy Products (in a ratio of 93% milk – 5% yoghurt – 2% cheese) in an acidogenic bioreactor prior to its co-digestion with agroindustrial wastes. A continuously stirred tank reactor (CSTR) was used under mesophilic conditions (37°C) and Hydraulic Retention Time (HRT) of 6 days in order to enhance acidogenesis and bio-hydrogen production. CSTR experiments were performed to investigate the effect of controlled pH (4.0, 4.5, 4.7, 5.0, 5.3, 5.7), using a solution of 6N NaOH and KOH, on the production of bio-hydrogen and Volatile Fatty Acids (VFAs). The maximum hydrogen yield and productivity (of 1.27 mol H_2/mol carbohydrates consumed and 0.78 $L/L_R \cdot d$ respectively) as well as the maximum VFAs concentration(25.5 g/L) was obtained at pH 5.0, whereas the greatest degradation of carbohydrates was observed at pH 4.0 with the lowest biogas and hydrogen production. Acetic, butyric and propionic acid were the main organic metabolites detected, while lactic acid was identified as a major intermediate metabolite of the studied process which presented an intense accumulation prior to its conversion to butyric and/or acetic acid and hydrogen.

Keywords: End-of-Life Dairy Products, anaerobic digestion, acidogenesis, biohydrogen.

1. Introduction

Dairy farms and cheese factories are agro-industries that represent a considerable share of the worldwide economy with particular interest focused in the Mediterranean region. Currently, large quantities of expired (End-of-Life) dairy products (EoL-DPs) are disposed of in landfills despite the fact that landfilling of these easily biodegradable products does not comply with the 1999/31/EU Landfill directive which poses strict limits for the disposal of biodegradable wastes.Uncontrolled handling or insufficient treatment and disposal of these highly polluting organic agro-wastes encounters an imminent danger to the environment and public health.

EoL-DPsare associated with high biological (BOD_5) and chemical oxygen demand (COD), since they contain a significant amount of carbohydrates, mainly lactose (45–50 g/L), proteins (6–8 g/L), lipids (4–5 g/L) and mineral salts (8–10% of dried extract). Despite the high carbohydrate content of EoL-DPs, which issuitable for biological processing, their biologicaltreatment is quite problematic due to theirlow bicarbonate alkalinity, high COD concentration (up to 200 g COD/L) and tendency for rapid acidification.EoL-DPs represent, however, a potential renewable energy source, which could benefit society with a clean fuel in the form of biogas. Anaerobic biological processes such as anaerobic digestion (AD) of EoL-DPs and other organic-based waste products could be a solution to the management of these wastes through simultaneous production of bioenergy and reduction of greenhouse gas emissions (Cantrell et al., 2008).

During the first stage of anaerobic digestion(acidogenesis) several bioprocesses may take placeincluding hydrolysis, acidification and H_2 gas production. Since a lot of different types of bacteria are involved in

the fermentation of organic wastes, anaerobic acidogenesis of this kind of wastes may produce volatile fatty acids (VFAs), alcohols, H_2, CO_2 and other intermediate products (i.e. lactic acid). Although pure carbohydrates (e.g. glucose, sucrose) have been most commonly used for H_2production (Wang and Wan, 2009), many different substrates such as solid wastes and food industry wastewaters can be also easily fermented (Kapdan and Kargi, 2006).

A series of operating parameters including pH, temperature and hydraulic retention time are known to influence the performance of AD and the formation of intermediate fermentative products such as H_2, organic acids and ammonia (Wanfigur and Wan, 2009). Among these factors, Ren et al. (1997) and Chen et al. (2002) presented that pH could be crucial to the distribution of acidogenic products. Ahuge number of studies have been conducted on the optimal pH range for fermentative H_2 production, but the results are often different due to differences in substrates and other operating conditions(Luo et al., 2010;Wu et al. 2010). Furthermore, many studies have been focused on the effect of initial pH on fermentative H_2 production, whereas the importance of pH control has rarely been investigated (Wang and Wan, 2009). Meanwhile, the reported optimal pH values for different substrates differ substantially from 4.0 to 6.5, but for each specific situation, the optimal pH range was quite narrow (usually within 0.5). For instance, an optimal pH value of 6.0 was obtained using cheese whey (Ferchichi et al., 2005), a lower pH value of 5.5 was proposed as optimum using glucose (Fangand Liu, 2002), whereas an initial pH value of 4.5 was preferred using sucrose and starch as substrate (Khanalet al., 2004).

This study is focused on the anaerobic treatment of a mixture of EoL-DPs with a high organic content, consisting of 93% (w/w) milk, 5% (w/w) yoghurt and 2% (w/w) cheese (anari) in a CSTR anaerobic reactor. The specific aim of the present work was to study the effect of pH on H_2 production in a continuously operatingacidogenic bioreactor and more specifically to determine experimentally the optimum pH value and identify the main metabolic pathways followed for both H_2 and VFAs production.

The pH values tested were 5.7, 5.3, 5.0, 4.7, 4.5 and 4.0 and were maintained constant throughout each experimental run.

2. Materials and Methods

2.1. Feedstock

The raw materials used in the present study included End-of-Life Dairy Products (EoL-DPs) (milk, yogurt and cheese“anari”) produced by a dairy industry located in Cyprus. In order to maintain the wastes’ characteristics as constant as possible, all wastes were stored immediately after sampling in the freezer at -18 °C until subsequent use throughout the experimentation period. The composition of the mixture of EoL-DPs, used in the acidogenic CSTR feed was estimated taking into account their type (milk, yogurt, cheese) and their fat content (i.e. full-fat, light and skimmed products), according to the official inventory data provided by the producing dairy company. The resulting composition of EoL-DPs was 93% milk – 5% yogurt – 2% cheese and is presented in detail in Table 1.

Table 1: Composition of EoL-DPs mixture

EoL-DPs mixture		
93% Milk	**5% Yoghurt**	**2% Cheese**
29% Full – fat	0.9% Full – fat	
52% Light	2.1% Light	2% Anari
12% Skimmed	2% Skimmed	

2.2. Experimental setup

Anaerobic digestion experiments were carried out in an acidogenic CSTR reactor. The bioreactor was cylindrical in shape, made entirely of stainless steel (INOX 316), having a double wall and working volume of 750 mL and was operated at constant temperature of 37 ± 0.2 °C via a thermocouple controller.

Agitation was performed by a geared motor drive unit which was installed on the top of reactor. The feedstock was stored in a tank placed in a refrigerator to maintain constant temperature at 4°C. Biogas production was measured by an automatic tailor-made device comprising of a combination of an engine oil filled U-tube, an electron – valve and a counter. The measurement was based on counting the number of displacements of constant oil volume by the produced biogas in biogas line. Anaerobic conditions in the anaerobic bioreactor were ensured by sparging with N_2 gas its liquid content at the beginning of experimentation. Aliquots of mixed liquor (effluent) samples were withdrawn and analyzed at least in duplicate for monitoring the reactor's performance under continuous stirring conditions.

Based on the design of this system the mixture of EoL-DPs was directly fed in the acidogenic reactor which was operated at Hydraulic Retention Time of six days (HRT6d). Operation at lower acidogenic HRTs hadresulted to accumulation of lactic acid and inhibition of the fermentation process.Each pH value tested in the acidogenic reactor was kept constant throughout the experimentation phase via automatic control (using a Hach PID-controller) by the addition of a solution mixture of NaOH (6 N) and KOH (6 N) or HCl 6N via a peristaltic pump.

For the start-up of the system, the acidogenic reactor was fed with sludge produced from an acidogenic reactor treating Olive Mill Wastewater(OMW), Cheese Whey (CW) and Liquid Cow Manure (LCM) in composition of 55%-40%-5%(w/w) respectively.

2.3. Analytical methods

Physicochemical analysis of samples and characterization of the EoL-DPs mixture included determination of the following parameters: pH, total and dissolved Chemical Oxygen Demand (tCOD and dCOD respectively), Total (TS) and Volatile (VS) Solids, Total (TSS) and Volatile (VSS) Suspended Solids, Volatile Fatty Acids (VFA), lactic acid, total (tCH) and dissolved (dCH) Carbohydrates, Total (TOC) and

dissolved (d-OC) Organic Carbon, Total Kjeldahl Nitrogen (TKN), ammonium Nitrogen (NH_4^+-N), total and ortho – Phosphates. All aforementioned determinations were carried out according to the *Standard Methods*.pH was measured using an electrode (Orion 3-Star). For the determination of carbohydrates, a colored sugar derivative was produced through the addition of L-tryptophan, sulfuric and boric acid, which was subsequently measured colorimetrically in a Cary 50 UV/VIS spectrophotometer (Varian) at the wavelength of 520 nm (Joseffson, 1983). For the quantification of Volatile Fatty Acids (VFA) 1 mL of sample was acidified with 30μL of 20% H_2SO_4 and then centrifuged (>3000 rpm) for 15 min to remove the biomass. The supernatant was filtered through a 0.22μm nylon filter and transferred to 2 mL septum-capped vials and analyzed in a gas chromatograph (Agilent Technologies 7890A) equipped with a flame ionization detector (FID). The oven temperature was gradually increased from 110^o C (held for 5 min) to 250^{o}C (held for 5 min) at a rate of 15^o C/min. Helium was used as the carrier gas at a flow rate of 15 mL/min. The injector temperature was set at 175^{o}C and the detector at 300^{o}C. A capillary column (DBFFAP, 30 m in length, 0.25 mm I.D. and 0.25μm packing film) was used for determining the concentration of the individual volatile fatty acids, i.e. acetic, propionic, isobutyric, butyric, isovaleric and valeric acid, which were detected at 3.8, 5.2, 5.7, 6.6, 7.2 and 8.0 min, respectively. Lactic acid in the culture medium was measured with a DIONEX IC300 ion chromatography system using a thermostated (30ºC) Dionex IonPac analytical column (AS19 length 4x250 mm and 7.5 mm I.D) and a guard column (4x50 mm length and 12 mm I.D.) and an electron conductivity detector (Dionex). Analysis was performed by elution gradient with KOH solution as mobile phase at flow rate of 0.8 mL/min. The eluent gradient was programmed to result in a 3 mM KOH solution during equilibration and analysis and a 70 mM KOH solution during column regeneration. The total running time of analysis was 28 min, and the gradient programme was scheduled as follows: 3 mM KOH for 14 min, 70 mM KOH in 3 min and maintained for 4 min and 3 mM KOH in 0.5 min until the end of running (28 min). The injection volume was 10 μL. The Total Organic Carbon (TOC) concentration was determined using a Shimadzu TOC

analyzer equipped with modules for the analysis of both liquid and solid samples. The gas volumes, produced from each experiment, were converted tostandard temperature (0 ºC) and pressure (760 mm Hg).

3. Results and discussion

3.1. Chemical composition of substrates

Physicochemical analysis of each EoL-DP waste stream and their mixture was performed (Table 2, 3, 4 and 5).

Table2: Characterization of milk products included in the feedstock

Parameters	Units	Full Fat Milk	Light Milk	Skimmed Milk
pH	**-**	6.85	6.82	6.73
TS	**g/L**	97.96	96.01	79.88
VS	**g/L**	88.65	88.65	72.46
SolubleCOD	**g/L**	102.21	85.51	97.68
Total COD	**g/L**	190.81	143.11	101.32
Total Organic C	**g/L**	71.39	56.02	46.18
Soluble Organic C	**g/L**	30.55	29.14	32.56
Total CH	**g/L**	44.17	44.57	48.37
TKN	**g/L**	2.89	4.00	3.23
NH_3-N	**g/L**	0.43	0.44	0.42
SolubleP	**mg/L**	469.71	605.48	641.94

Table3: Characterization of yoghurt products included in the feedstock

Parameters	Units	Full Fat Yoghurt	Light Yoghurt	Skimmed Yoghurt
pH	**-**	4.19	4.28	4.14
TS	**g/g fresh weight**	0.226	0.212	0.173
TS	**g/L**	234.59	220.06	179.57
VS	**g/g dry weight**	0.955	0.943	0.923
VS	**g/L**	224.03	207.51	165.75
SolubleCOD	**g/L**	66.70	89.37	85.32
Total COD	**g/100g fresh weight**	36.48	27.25	24.02
Total COD	**g/L**	378.66	282.86	249.33
Total Organic C	**g/100 g dry weight**	65.07	59.39	57.63
Total Organic C	**g/L**	152.65	130.69	103.49

Soluble Organic C	**g/L**	25.89	32.9	36.33
Total CH	**g/100 g dry weight**	15.45	18.98	27.73
Total CH	**g/L**	36.24	41.77	49.79
TKN	**g/100 g dry weight**	2.77	3.06	4.12
TKN	**g/L**	6.50	6.73	7.40
NH_3-N	**g/100 g dry weight**	0.41	0.56	0.58
NH_3-N	**g/L**	0.96	1.23	1.04
SolubleP	**mg/L**	1060	1329	1230

Table 4: Characterization of cheese products included in the feedstock

Parameters	Units	Fresh Anari	Unsalted Anari	Salted Anari
pH	**-**	4.92	5.46	5.37
TS	**g/g fresh weight**	0.209	0.265	0.279
TS	**g/L**	209.42	265.53	279.56
VS	**g/g dry weight**	0.949	0.936	0.945
VS	**g/L**	198.74	248.54	264.18
SolubleCOD	**g/100 g dry weight**	28.71	27.04	20.15
Soluble COD	**g/L**	60.12	71.80	56.33
Total COD	**g/100g fresh weight**	30.24	20.70	30.32
Total COD	**g/L**	303.00	207.41	303.81
Total Organic C	**g/ 100g dry weight**	55.30	68.31	66.70
Total Organic C	**g/L**	115.81	181.38	186.47
Soluble Organic C	**g/ 100g dry weight**	11.42	10.61	8.35
Soluble Organic C	**g/L**	23.92	28.17	23.34
Total CH	**g/100 g dry weight**	8.56	8.96	6.84
Total CH	**g/L**	17.93	23.79	19.12
TKN	**g/100 g dry weight**	5.07	3.95	4.61
TKN	**g/L**	10.62	10.49	12.89
NH_3-N	**g/100 g dry weight**	0.66	0.58	0.59
NH_3-N	**g/L**	1.38	1.54	1.65
SolubleP	**g/100g dry weight**	31.29	44.46	29.48
Soluble P	**g/L**	65.52	118.05	82.41

Table5: Characterization of the EoL-DPs mixture (93% milk- 5% yoghurt- 2% cheese)used as feedstock for the acidogenic experiments in this work.

Parameters	Units	Dairy Mixture (EoL-DPs)
pH	-	6.23
TSS	g/L	79.16
VSS	g/L	74.9
TS	g/L	121.54
VS	g/L	112.88
Total COD	g/L	188.36
Soluble COD	g/L	129.90
Total CH	g/L	47.23
TKN	g/L	3.88

NH_3-N	g/L	0.49
Total P	mg/L	990.00
Soluble P	mg/L	430.00

The dairy products were categorized in terms of their fat content, i.e. as full-fat, light and skimmed products, according to the official inventory of returns kept by the producing dairy industry. All types of EoL-DP wastes were of high organic load concentration and were characterized by their high content in carbohydrates. Tables 2, 3 and 4 present the characteristics of each dairy product while Table 5 shows the chemical composition of the dairy mixture used in this study.

1.1. Effect of pH on the anaerobic acidogenesis of EoL-DPs mixture for maximization of hydrogen production.

CSTR experiments were carried out using a mixture of End of Life dairy products at a ratio of 93% milk – 5% yoghurt – 2% cheese (anari) in order to assess the effect of pH on hydrogen production and distribution of products. All experiments were conducted in the same reactor configuration (Section 2.2.). During the reactor startup the system was inoculated with acclimatized anaerobic sludge, which was obtained from an anaerobic acidogenic CSTR digester fed daily with a mixture of OMW, CW and LCM at a ratio of 55% - 40% - 5% (w/w) and operated at steady-state conditions at hydraulic retention time (HRT) of 3 days under mesophilic conditions (37^{o}C). The pH values tested were 5.7, 5.3, 5.0, 4.7, 4.5 and 4.0. The pH of the mixed liquor was kept constant throughout the course of the experiment via automatic control (using a HACH PID-controller) by adding NaOH - KOH or HCl solution (6N) via respective peristaltic pumps. The quantity of produced biogas and its composition was monitored throughout the course of each experiment. At regular intervals samples were collected for analysis, i.e. determination of carbohydrates, VFAs, lactic acid, TS, VS.

Figure 1b illustrates the quantity of biogas and hydrogen produced at STP conditions, the total sugars consumption and the main metabolic end-products, as function of pH. In all pH values tested, the biogas was mainly composed of hydrogen (H_2) and carbon dioxide (CO_2), whereas the mixed liquor was composed of VFA and lactic acid. As shown in Figure 1b the biogas and hydrogen production were pH dependent. The maximum production of hydrogen (780 mL $H_2/L_R \cdot d$) was obtained at pH 5.0, whereas increasing or lowering the pH value resulted to lower hydrogen productivity. During all fermentation experiments, no methane production was detected indicating that only acidogenesis was active. The consumption of total carbohydrates, measured as equivalent glucose, (Figure 1a) was high in all tested pH values with the maximum degradation (94.1%) observed at pH 4.0. Similarly, soluble carbohydrates consumption was even higher (98.9%) at pH 4.7 despite their simultaneous production due to hydrolysis of total carbohydrates. Ferchichiet al. (2005) observed equally high sugar consumption (97%) studying hydrogen production from cheese whey at different initial pH values ranging from 5.0 to 9.0, suggesting that the microorganisms' ability to consume sugars did not alter within this initial pH range.

Degradation of glucose under anaerobic conditions is accompanied by production of hydrogen and various metabolic products, mainly volatile fatty acids (i.e. acetic, propionic, and butyric acids), lactic acid and alcohols (ethanol), depending on the microbial species present and the prevailing conditions. The analysis of metabolic products provides useful information on the evolution of the process and can be used to explain the observed hydrogen generation yields. In the present study, the course of soluble metabolites' concentration was monitored during the process. A mixture of acetic, propionic, butyric and lactic acid was measured as abundant metabolites which are characteristic of *clostridia* fermentation. The highest total VFAs concentration was detected at pH 5.0 (25.5 g/L) withthe concentrations of acetic and butyric acid being4.7 g/L and 15.8 g/L, respectively. Zhanget al. (2013)reported that acetic acid was the main product at pH 5.0, whereas butyric acid was dominant at pH 6.0, during anaerobic acidogenesis of kitchen wastes. Limited amounts (<400 mg/L) of other volatile fatty acids (i.e. i-butyric, valeric, i-valeric

and caproic) were detected in all pH values tested in this study. Table 6 presents additional results, including solids' degradation, obtained during the tests carried out in this work. Apart from the highest carbohydrates' degradation, the maximum removal of total and volatile solids (i.e. 39.4% and 52.6%, respectively) occurred at pH 5.0. Similarly, Zhanget al. (2013) observed also that thegreatest degree of hydrolysis and acidogenesis accompanied the maximum hydrogen production and VFAs concentration.

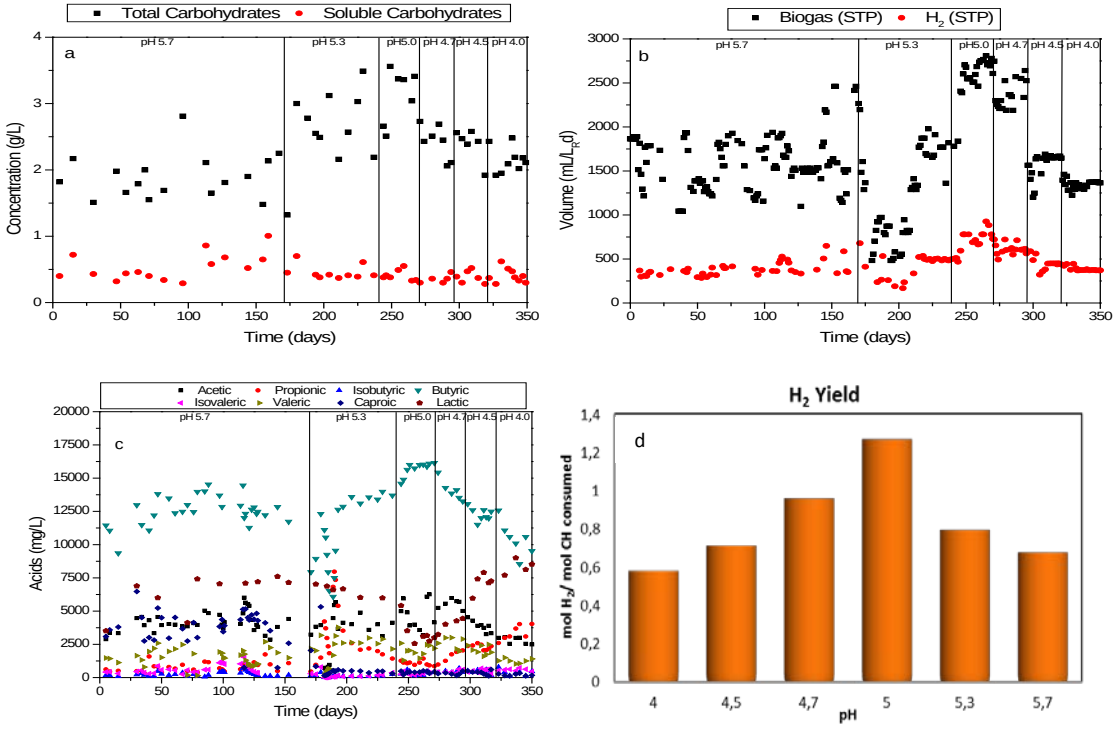

Fig.1: Performance of the acidogenic reactor versus experimental time for each pH: (a) effluent concentrations of total and soluble carbohydrates, (b) biogas and hydrogen production rates (c) effluent concentrations of VFAs and lactic acid and (d)hydrogen yields.

In particular, the consumption of total and soluble sugars is associated with the rate of production of major products during the whole experimentation course. The degradation of carbohydrates (Figure 1a) contributed to an increase of the concentration of acetic and lactic acid (Figure 1c). Significant accumulation of butyric acid (15.8g/L) was observed mainly as a result of lactic acid degradation, which was also accompanied by simultaneous decrease in acetic acid and increase in production of hydrogen. Furthermore, propionic acid was found to be produced in appreciable amounts only at later fermentation stages (i.e., when decrease of concentration of lactic acid was observed). Matsumoto and Nishimura (2007) found that a mixed substrate of acetic and lactic acid enhanced hydrogen production by strain *Clostridium diolis* JPCC H-3 and it was also observed that 1 mol of acetic acid reacted with 2 mol of lactic acid and produced 1 mol of H_2, 2 mol of CO_2, and 1.5 mol of butyric acid.

Figure 1d and Table 6 depict the hydrogen yield (moles of hydrogen produced per mole equivalent glucose of total consumed carbohydrates) at each pH value tested. The maximum hydrogen yield was observed at pH 5.0 and was equal to 1.27 molH_2/mol equivalent glucose consumed. According to literature, the optimum hydrogen yield should be achieved with acetic acid as the fermentation end-product (theoretical yield of 4 mol of H_2/mol of glucose). However, in our caseno hydrogen seemed to be produced along with acetic acid in pH 5.0, whereas hydrogen productivity seemed to be closely related to butyric acid production and lactic acid degradation.We consider that hydrogen production decrease in both lower and higher pH values than 5.0 is mainly due to enzymatic inhibition and not simultaneous consumption of produced hydrogen, since no methane was detected in all experiments.

Lactic acid was identified as a major intermediate soluble product in all fermentation processes, since it was firstly produced and subsequently metabolized during the process at a greater or lower extent depending on the applied pH. For instance, in pH 5.0, lactic acid started to degrade until it was detected at a concentration of 3.0 g/L at the end of the CSTR test (Figure 1c).More specifically, accumulation of lactic acid was observed at both low and high pH values due to kinetic limitation in the reactions

converting lactic acid to butyric acid and hydrogen. Lactic acid bacteria, including species of the *Lactobacillus* genus, are naturally found in dairy products as a result of the milk and cheese making process.*Lactobacilli* produce lactic acid as the major fermentation product from sugars (Stiles and Holzapfel, 1997).

Table 6: Analysis of acidogenic experiments (reactor performance) for HRT 6d and each pH value in steady state operation

	Biogas (STP) ($LL_r^{-1}d^{-1}$)	**H_2 (STP) ($LL_r^{-1}d^{-1}$)**	**Biogas (STP) (LL_f^{-1})**	**H_2 (STP) (LL_f^{-1})**	**$\%H_2$**	**H_2 Yield ($molH_2$/ molCH cons.)**	**Degradation**			
							%t-CH	**%d-CH**	**% TS**	**%VS**
pH 4.0	1.343	0.371	8.06	2.23	27.6	0.580	94.1	98.3	16.1	25.7
pH 4.5	1.647	0.450	9.88	2.70	27.3	0.712	93.0	97.8	26.4	37.6
pH 4.7	2.314	0.607	13.89	3.65	26.3	0.959	93.2	98.9	35.8	44.9
pH 5.0	2.659	0.780	15.96	4.68	29.3	1.268	90.6	98.6	39.4	52.6
pH 5.3	1.772	0.500	10.63	3.00	28.2	0.794	92.7	98.5	28.9	42.4
pH 5.7	1.908	0.388	11.45	2.33	20.3	0.676	90.9	96.7	26.0	39.2

The appearance of lactic acid has also been observed with other carbohydrate-rich substrates like potato waste (Parawira et al., 2004) and garbage(Akao et al., 2007). According toCastellóet al. (2009), *Lactobacilli* are capable of producing lactic acid from lactose via three metabolic pathways, i.e. the homofermentative (Equation 1a), the heterofermentative (Equation 1b) and the bifidum pathway (Equation 1c):

Homofermentative pathway

$C_6H_{12}O_6 \rightarrow 2CH_3CH(OH)COOH$ (Eq. 1a)

Heterofermentative pathway

$C_6H_{12}O_6 \rightarrow CH_3CH(OH)COOH + CH_3CH_2OH + CO_2$ (Eq. 1b)

Bifidum pathway

$2C_6H_{12}O_6 \rightarrow 3CH_3COOH + 2CH_3CH(OH)COOH$ (Eq. 1c)

In general, hydrogen yields vary proportionally to the final metabolic products. Acetic and butyric acid favor the production of hydrogen with the fermentation of glucose to acetic acid giving the highest theoretical yield of 4 mol of H_2/mol of glucose (Equation2) whileglucoseconversion to butyric acid results to 2 mol of H_2/mol of glucose (Equation 3). However, lactic acid is not accompanied by hydrogen generation (Antonopoulou et al., 2008).

$C_6H_{12}O_6 + 2H_2O \rightarrow 2CH_3COOH + 2CO_2 + 4H_2$ (Eq. 2)

$C_6H_{12}O_6 \rightarrow CH_3CH_2CH_2COOH + 2CO_2 + 2H_2$ (Eq.3)

Because of the complexity of microbial populations present in the mixed culture used in our experiment, a combination of fermentative pathways was assumed to take place. Initially the conversion of glucose to lactic acid and acetic acid takes place (Eqs. 1a - 1c). However, the contribution of each pathway (Eqs. 1a –1c) to the production of intermediates was impossible to be clarified at this point. Butyric acid was detected as the main final volatile fatty acid. The increased hydrogen production was observed that is primarily associated with the consumption of lactic acid with simultaneous production of butyric acid and hydrogen according Equation 3. At lower and higher pH values of 5.0 hydrogen productivity decreased due to lactic acid kinetic limitation. Our results indicate that pH is a very important factor because of the limitation of hydrogen production outside a narrow pH range.Mohd Yasinet al. (2011) reported that anaerobic fermentation of food waste at thermophilic conditions was suitable for bio-hydrogen production at controlled pH 5.5 with a yield of 79 mmol H_2/L-medium/d. A range of optimum pH values has been reported in the literature for fermentation of carbohydrates by mixed bacterial cultures. Van Ginkelet al.

(2001)for instance, studied the effect of varying pH (4.5–7.5) and demonstrated that the highest hydrogen production rate (74.7 mL H_2/L·h) occurred at pH 5.5 and substrate concentration of 7.5 g COD/L. Davila-Vazquezet al. (2008) studied the effect of the initial pH (3.88–8.12) using glucose, lactose and cheese whey powder as substrates, with the highest hydrogen yield being observed from glucose and lactose at an initial pH 7.5 and from cheese whey powder at an initial pH value of 6.0. Another study by Fangand Liu (2002), using glucose at varying pH values from 4.0 to 7.0 reportedthat their maximum yield was2.1 ± 0.1 mol H_2/mol glucose and was observed at pH 5.5.

It should also be noted that the comparison of the pH effects on hydrogen production reported in the present study and those in the literature is complicated by the fact that most of the latter report theirexperimental results for runs where only the initial pH was adjusted [11, 25] without any further control along the process. Therefore, it can be concluded that the pH value during the process is a very crucial parameter for the main metabolic products evolution and particularly for hydrogen production.

2. Conclusions

Based on the obtained results of this work, the optimum conversion of EoL-DPsto hydrogen and the maximum hydrogen yield of 1.27 moles H_2/ moles equivalent glucose was observed at pH 5.0. The biogas produced from the acidogenic reactor consisted exclusively of hydrogen and carbon dioxide and was free of methane. The hydrogen production at pH 5.0 was fluctuating with a mean value of 0.780 L $L^{-1}_{reactor}$ d^{-1} at steady state. Lactic acid was detected as the main intermediate acid, while the butyric acid wasthe main final volatile fatty acid. The increased hydrogen production was primarily associated with the consumption of lactic acid with simultaneous production of butyric acid and hydrogen.

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